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CLINICAL THERAPEUTICS

Clinical Impact of the Revised 2019 CLSI Levofloxacin Breakpoints in Patients with *Enterobacterales* Bacteremia

Ho-Yin Huang,[a,b] Chu-Feng Wang,[c] Po-Liang Lu,[d,e] Sung-Pin Tseng,[f] Ya-Ling Wang,[a] Tun-Chieh Chen,[d,g] Ko Chang,[d,h] Hung-Pin Tu,[i] Shang-Yi Lin[c,d,e,j]

[a]Department of Pharmacy, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[b]School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan
[c]Department of Laboratory Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[d]Division of Infectious Diseases, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[e]College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[f]Department of Medical Laboratory Science and Biotechnology, College of Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan
[g]Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[h]Department of Internal Medicine, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[i]Department of Public Health and Environmental Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[j]Graduate Institute of Clinical Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

ABSTRACT The Clinical and Laboratory Standards Institute (CLSI) revised the fluoroquinolone MIC breakpoints for *Enterobacterales* in 2019, based on pharmacokinetic/pharmacodynamic analyses. However, clinical evidence supporting these breakpoint revisions is limited. A retrospective study was conducted at 3 hospitals in Taiwan between January 2017 and March 2019. Patients treated with levofloxacin for bacteremia caused by members of the *Enterobacterales* with high MICs (1 or 2 μg/ml; levofloxacin susceptible by pre-2019 CLSI breakpoints) were compared with those with low-MIC bacteremia ($\leq$0.5 μg/ml; levofloxacin susceptible by 2019 CLSI breakpoints) to assess therapeutic effectiveness by multivariable logistic regression. The primary outcome was 30-day mortality, and the secondary outcome was the emergence of levofloxacin-resistant isolates within 90 days after levofloxacin initiation. A total of 308 patients were eligible for the study. Kaplan-Meier analysis showed that patients infected with high-MIC isolates ($n = 63$) had a significantly lower survival rate than those infected with low-MIC isolates ($n = 245$) ($P = 0.001$). Multivariable logistic regression revealed that high levofloxacin MIC was a predictor of 30-day mortality (odds ratio [OR], 6.05; 95% confidence interval [CI], 1.51 to 24.18; $P = 0.011$). We consistently found similar results in a propensity score-matched cohort (OR, 5.38; 95% CI, 1.06 to 27.39; $P = 0.043$). The emergence of levofloxacin-resistant isolates was more common in the high-MIC group than the low-MIC group (25.0% versus 7.5%; $P = 0.065$). An estimated area under the concentration-time curve/MIC ratio of $\geq$87 was significantly associated with better survival ($P = 0.002$). In conclusion, patients infected with isolates with levofloxacin MICs within the pre-2019 CLSI susceptible range of 1 or 2 μg/ml exhibited higher mortality than those infected with isolates with MICs of $\leq$0.5 μg/ml.

KEYWORDS levofloxacin, MIC, breakpoint, CLSI, *Enterobacterales*

Levofloxacin is a broad-spectrum fluoroquinolone (FQ) antibiotic that is used to treat a wide range of infections caused by Gram-negative and Gram-positive bacteria (1). FQ inhibits the activity of DNA gyrase and topoisomerase IV, which are necessary for bacterial DNA replication, transcription, repair, and recombination (2). However, increasing use of FQs over the past 20 years has led to the emergence of FQ-resistant

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Address correspondence to Shang-Yi Lin, amoe616@gmail.com.

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organisms and thus reduced their therapeutic effectiveness, which has in turn become an important public health concern (3).

In 2019, the Clinical and Laboratory Standards Institute (CLSI) revised and lowered the levofloxacin MIC breakpoints for *Enterobacterales* (4). The updated antimicrobial susceptibility testing (AST) criteria refer to MICs of $\leq$0.5 μg/ml as susceptible, MICs of 1 μg/ml as intermediate, and MICs of $\geq$2 μg/ml as resistant to levofloxacin, in contrast to the previous criteria, where MICs of $\leq$2 μg/ml were considered susceptible, MICs of 4 μg/ml were intermediate, and MICs of $\geq$8 μg/ml were resistant (5). This breakpoint revision was based on pharmacokinetic (PK)/pharmacodynamic (PD) analyses in combination with Monte Carlo simulations, which indicated that the previous breakpoints were too high; in addition, a levofloxacin dosage regimen of 750 mg every 24 h is recommended in the CLSI 2019 breakpoints for *Enterobacterales*, whereas there were no dosages specified in the pre-2019 criteria (5).

There have been only a few previous studies assessing the impact of levofloxacin MICs on clinical outcomes, and these have had inconsistent results (6–8). Whether these revised breakpoints are too liberal or too conservative with respect to clinical outcomes remains to be determined. The present study investigated whether *Enterobacterales* bacteremia isolates that were previously identified as levofloxacin susceptible and have now been reclassified as levofloxacin intermediate or levofloxacin resistant by the 2019 CLSI criteria are associated with higher mortality than isolates that were identified as levofloxacin susceptible by both criteria.

RESULTS

***Enterobacterales* isolates whose susceptibility status changed between the pre-2019 and 2019 CLSI breakpoints.** During the study period, a total of 4,037 patients with bacteremia due to *Enterobacterales* were identified, and 3,131 (77.6%) isolates were identified as being in the susceptible category with MICs of $\leq$2 μg/ml. According to the updated 2019 CLSI levofloxacin breakpoints, 15.1% of isolates previously considered susceptible were reclassified; 14.1% of isolates formerly identified as levofloxacin susceptible were reclassified as levofloxacin intermediate, while 1.0% were reclassified as levofloxacin resistant (see Fig. S1 in the supplemental material). The quarterly levofloxacin usage and resistance density of *Enterobacterales* stayed stable from 2017 to the first quarter of 2019 (Fig. S2). There were no significant correlations over time for levofloxacin consumption with levofloxacin resistance defined by the pre-2019 (ρ = $-$0.34 and P = 0.375) or 2019 (ρ = $-$0.39 and P = 0.305) CLSI criteria.

Patient demographics, clinical characteristics, and outcomes. Of the 308 patients who met the eligibility criteria, 245 were in the low-MIC group ($\leq$0.5 μg/ml) and 63 were in the high-MIC group (1 or 2 μg/ml) (Fig. 1). The characteristics and outcomes of patients are shown in Table 1. Infections were community acquired in the majority of cases, as opposed to hospital acquired (21.4%). The common sources of bacteremia were urinary tract infections (69.8%), intra-abdominal infections (12.7%), and pneumonia (10.1%). *Escherichia coli* (60.7%) was the predominant organism isolated from blood cultures, followed by *Klebsiella* spp. (19.8%) and *Enterobacter* spp. (9.4%). The proportion of patients with a complex comorbidity status (Charlson comorbidity index [CCI] $\geq$ 3), high Pitt bacteremia score ($\geq$4), and hospital-acquired infection were significantly higher in the high-MIC group. The proportion of patients who received initial intravenous levofloxacin therapy was similar between two groups (207/245 [84.5%] versus 54/63 [85.7%]; P = 1.000). The median duration of levofloxacin therapy was 10 days (interquartile range, 7 to 12.75). Eighty-three patients (26.9%) had blood cultures within 90 days after levofloxacin initiation, and 18 of 83 patients (21.7%) had blood cultures positive for *Enterobacterales* isolates. The emergence of levofloxacin-resistant isolates was more common in the high-MIC group than the low-MIC group (25.0% versus 7.5%; P = 0.065). In total, 12 patients (3.9%) died within 30 days of their bacteremia diagnosis. The Kaplan-Meier plot revealed a significantly higher survival rate in the low-MIC group than the high-MIC group (log-rank test, P = 0.001) (Fig. 2).

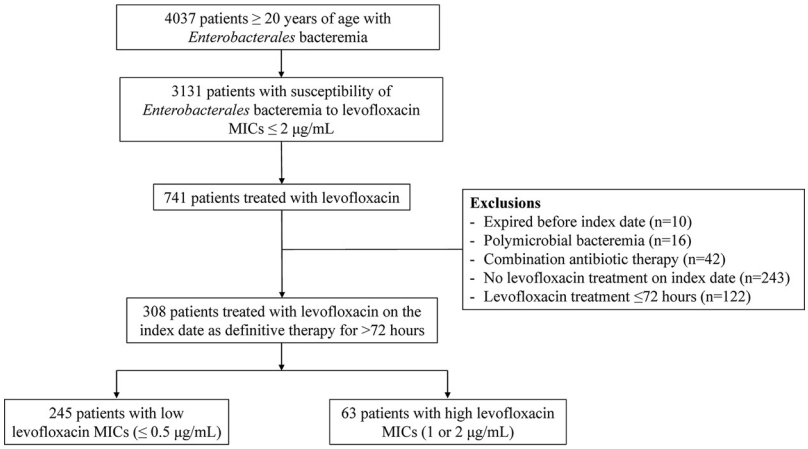

FIG 1 Flowchart of patient selection.

In the multivariable logistic regression, high levofloxacin MIC, a CCI of ≥3, intensive care unit (ICU) admission, and urinary tract source were independently associated with 30-day mortality (Table 2). Given that more patients received nonactive empirical antibiotic therapy in the high-MIC group, with marginal significance ($P = 0.063$), a sensitivity analysis including this variable was performed and showed the same final regression model. We consistently found that the high-MIC group was associated with higher 30-day mortality than the low-MIC group in 181 propensity score-matched patients (odds ratio [OR], 5.38; 95% confidence interval [CI], 1.06 to 27.39; $P = 0.043$) (see Table S1).

PK/PD target attainment on clinical outcomes. A dot plot shows the PK/PD analysis of the estimated levofloxacin area under the concentration-time curve (AUC)/MIC ratio in the low- and high-MIC groups for 308 patients (Fig. 3A). The mean dosage of levofloxacin was comparable in the high- and low-MIC groups (638.89 ± 15.78 mg versus 668.37 ± 7.52 mg; $P = 0.081$). The calculated AUC ranged from 14.50 to 2,140.99 mg·h/liter (195.52 ± 30.59 mg·h/liter in the high-MIC group versus 215.35 ± 19.95 mg·h/liter in the low-MIC group; $P = 0.458$). The estimated AUC/MIC was significantly lower in the high-MIC group than the low-MIC group (180.43 ± 27.93 versus 1,671.26 ± 152.62; $P < 0.001$). Figure 3B shows that the ratio of estimated levofloxacin AUC to MIC of ≥87, which was used to represent the clinical total-drug AUC/MIC ratio target for efficacy for *Enterobacterales* (9), was associated with significantly better survival ($P = 0.002$).

DISCUSSION

The present multicenter, retrospective cohort study revealed that there was a significantly increased risk of 30-day mortality for patients with *Enterobacterales* bacteremia caused by organisms with MICs of 1 or 2 μg/ml that were previously recognized as levofloxacin susceptible and are now reclassified as levofloxacin intermediate or levofloxacin resistant according to the 2019 CLSI criteria, compared with patients infected with bacteria with MICs of ≤0.5 μg/ml. Although there was no statistical significance, our study also suggests a trend toward an increased risk of harboring levofloxacin-resistant isolates within 90 days after antibiotic initiation in the high-MIC group compared to the low-MIC group. Additionally, better clinical outcomes were observed in the PK/PD target ratio of estimated AUC to MIC of ≥87.

Among all *Enterobacterales*, the magnitude of susceptibility decreased in a stepwise manner for levofloxacin accompanied by the growing prevalence of FQ resistance (10). The proportion of levofloxacin-susceptible strains within the range of MICs of ≤2 μg/ml declined from 97.4 to 69.9% in *E. coli* and from 95.8 to 87.0% in *Klebsiella pneumoniae*, respectively, between 1998 and 2013 (11). In the current study, 77.6% of

TABLE 1 Clinical characteristics and outcomes of 308 patients with *Enterobacterales* bacteremia received levofloxacin treatment

Characteristic[a]	Value (%)[b] for: All patients ($n = 308$)	Low-MIC patients ($n = 245$)	High-MIC patients ($n = 63$)	*P* value
Demographics				
Age, yr (mean ± SD)	67.32 ± 14.62	67.40 ± 14.69	67.02 ± 14.45	0.853
Sex, male	131 (42.5)	107 (43.7)	24 (38.1)	0.476
Comorbidities				
Cardiovascular disease	199 (64.6)	156 (63.7)	43 (68.3)	0.556
Chronic pulmonary disease	10 (3.2)	8 (3.3)	2 (3.2)	1.000
Chronic liver disease	14 (4.5)	10 (4.1)	4 (6.3)	0.496
Chronic renal disease	26 (8.4)	19 (7.8)	7 (11.1)	0.445
Diabetes	114 (37.0)	87 (35.5)	27 (42.9)	0.307
Malignancy	82 (26.6)	64 (26.1)	18 (28.6)	0.750
Immunocompromised status	28 (9.1)	21 (8.6)	7 (11.1)	0.622
CCI ≥3	114 (37.0)	83 (33.9)	31 (49.2)	0.029
Clinical severity				
ICU admission	36 (11.7)	26 (10.6)	10 (15.9)	0.272
SOFA score ≥5	61 (19.8)	45 (18.4)	16 (25.4)	0.218
Pitt bacteremia score ≥4	22 (7.1)	10 (4.1)	12 (19.0)	<0.001
Hospital acquired	66 (21.4)	43 (17.6)	23 (36.5)	0.002
Source of bacteremia				
Pneumonia	31 (10.1)	27 (11.0)	4 (6.3)	0.352
Intra-abdominal	39 (12.7)	33 (13.5)	6 (9.5)	0.525
Urinary tract	215 (69.8)	169 (69.0)	46 (73.0)	0.645
Skin and soft tissue	8 (2.6)	7 (2.9)	1 (1.6)	1.000
Catheter related	10 (3.2)	6 (2.4)	4 (6.3)	0.126
Others[c]	5 (1.6)	3 (1.2)	2 (3.2)	0.271
Organism				
Escherichia coli	187 (60.7)	145 (59.2)	42 (66.7)	0.313
Enterobacter spp.	29 (9.4)	25 (10.2)	4 (6.3)	0.471
Klebsiella spp.	61 (19.8)	49 (20.0)	12 (19.0)	1.000
Citrobacter spp.	11 (3.6)	11 (4.5)	0 (0.0)	0.129
Proteus spp.	11 (3.6)	8 (3.3)	3 (4.8)	0.702
Serratia marcescens	8 (2.6)	7 (2.9)	1 (1.6)	1.000
Providencia stuartii	1 (0.3)	0 (0.0)	1 (1.6)	0.205
Empiric therapy				0.063
Levofloxacin	148 (48.1)	123 (50.2)	25 (39.7)	
Nonactive antibiotic	61 (19.8)	42 (17.1)	19 (30.2)	
Active antibiotic	99 (32.1)	80 (32.7)	19 (30.2)	
Levofloxacin therapy				
750 mg	200 (64.9)	165 (67.3)	35 (55.6)	0.103
500 mg	108 (35.1)	80 (32.7)	28 (44.4)	
Source control[d]	85 (27.6)	68 (27.8)	17 (27.0)	1.000
Outcomes				
30-day mortality	12 (3.9)	5 (2.0)	7 (11.1)	0.004
Emergence of resistance	9/83[e] (10.8)	5/67 (7.5)	4/16 (25.0)	0.065

[a]CCI, Charlson comorbidity index; ICU, intensive care unit; SOFA, sequential organ failure assessment.
[b]Low MIC was defined as ≤0.5 μg/ml; high MIC was defined as 1 or 2 μg/ml.
[c]Other sources included endocarditis (one patient), central nervous system infection (one patient), and unknown sources (three patients).
[d]Source control was defined as a removable focus or resolution.
[e]Eighty-three patients had blood cultures within 90 days after levofloxacin initiation.

Enterobacterales isolates were identified as levofloxacin susceptible, with MICs of ≤2 μg/ml, while 20.3% were levofloxacin resistant, with MICs of ≥8 μg/ml. This was comparable with epidemiological evidence that 79.2% of *Enterobacterales* isolates from bloodstream infections were levofloxacin susceptible and 18.5% were

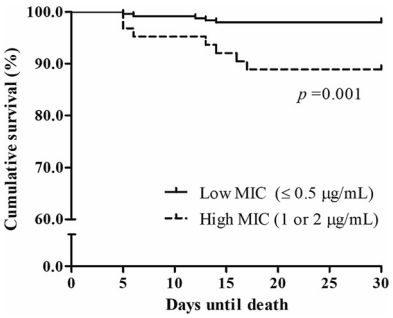

FIG 2 Kaplan-Meier survival analysis curve for patients infected with *Enterobacterales* isolates with low and high levofloxacin MICs.

levofloxacin resistant in the SENTRY Antimicrobial Surveillance Program in 1997 to 2016 (12). More recently, it was reported that fluoroquinolone consumption and levofloxacin-resistant *E. coli* were potentially associated on a nationwide scale in Japan from 2015 to 2016 (13). However, no correlation for quarterly data of levofloxacin-resistant *Enterobacterales* isolates with levofloxacin use was observed during our study period.

Given that levofloxacin displays a concentration-dependent and a prolonged post-antibiotic PD effect, the ratio of AUC to MIC was the PK parameter that appears to best correlate with clinical efficacy and microbiological response, which was the major impetus for the breakpoint revisions (14, 15). The commonly used levofloxacin dosing regimen of 750 mg every 24 h results in a ≥93.0% probability of attaining free-drug AUC/MIC ratio targets by MICs of ≤0.5 μg/ml, whereas probabilities are 42.7% and 61.5% for the oral and intravenous routes, respectively, for MICs of 1 μg/ml (11). It is suggested that the increasing resistance may have impacted the breakpoint revision. Using the updated 2019 CLSI breakpoints for FQs, the present study showed that the susceptibility of *Enterobacterales* to levofloxacin declined by 15.1% in Taiwan, and a 5.2% reduction in ciprofloxacin susceptibility was reported in a recent study in the United States (16). In addition to the potential adverse events associated with FQs (17), modification of the interpretive criteria has resulted in an increased proportion of strains being identified as resistant, which might restrict the prescription of FQs and thus improve antimicrobial stewardship.

Limited data are available to determine the impact of MICs within the susceptible range of *Enterobacterales* on clinical outcomes. In the current cohort, a high levofloxacin MIC of 1 or 2 μg/ml was identified as an independent predictor for 30-day mortality after adjustment for the potential cofounders of underlying comorbidities and infection severity, compared with MICs of ≤0.5 μg/ml. A recent study also demonstrated

TABLE 2 Univariable and multivariable logistic regression analyses of factors associated with 30-day mortality[a]

	30-day mortality		Univariable analysis			Multivariable analysis		
Variable	Survivors (n = 296)	Nonsurvivors (n = 12)	OR	95% CI	P	OR	95% CI	P
High LEV MIC (1 or 2 μg/ml)	56 (18.9)	7 (58.3)	6.00	1.84–19.60	0.003	6.05	1.51–24.18	0.011
Chronic liver disease	11 (3.7)	3 (25.0)	8.64	2.05–36.41	0.003			
Malignancy	75 (25.3)	7 (58.3)	4.13	1.27–13.39	0.018			
CCI ≥3	104 (35.1)	10 (83.3)	9.23	1.99–42.92	0.005	8.79	1.60–48.32	0.012
ICU admission	29 (9.8)	7 (58.3)	12.89	3.84–43.22	<0.001	6.47	1.58–26.50	0.009
SOFA score ≥5	53 (17.9)	8 (66.7)	9.17	2.66–31.58	<0.001			
Pitt bacteremia score ≥4	17 (5.7)	5 (41.7)	11.72	3.37–40.82	<0.001			
Pneumonia source	26 (8.8)	5 (41.7)	7.42	2.20–25.03	0.001			
Urinary tract source	213 (72.0)	2 (16.7)	0.08	0.02–0.36	0.001	0.11	0.20–0.60	0.011

[a]LEV, levofloxacin; CCI, Charlson comorbidity index; ICU, intensive care unit; SOFA, sequential organ failure assessment. Model adequacy was evaluated using the Hosmer-Lemeshow goodness-of-fit test (P = 0.963) and the area under the receiver operating characteristic (ROC) curve was 0.94 (95% CI, 0.90 to 0.99).

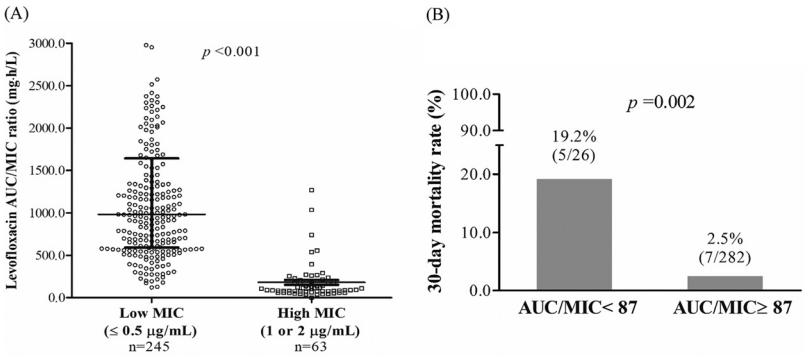

FIG 3 (A) Ratio of levofloxacin estimated AUC/MIC according to groups infected with isolates with low versus high MICs. Heavy black bars represent medians and interquartile ranges (23 data points are outside the axis limits in the low-MIC group). (B) Proportion of mortality according to target attainment of estimated levofloxacin AUC/MIC of ≥87. AUC/MIC, ratio of area under the concentration-time curve to MIC.

the negative impact of reduced FQ susceptibility on treatment responses in *E. coli* uropathogens (7). A meta-analysis concluded that high MICs of various antibiotics within the contemporary susceptible range were associated with a higher mortality rate but not with treatment failures for patients infected with *Enterobacterales* (18). More specifically, in patients treated with levofloxacin, a borderline significance and no between-group difference of all-cause mortality were observed for patients infected with *Enterobacterales* strains with MICs of 1 or 2 μg/ml compared with MICs of ≤0.5 μg/ml (6, 8). The overall 30-day mortality rate was 3.9% in the present study, which was comparable to the previously reported rates of 4.5% and 5.3% (6, 8). It has been suggested that this relatively low mortality rate in a small population of patients may make it difficult to determine the true effect. Since the definition of treatment failure and the study populations varied between studies, the robustness of these results remains uncertain. Notably, urinary tract infection as the source of bacteremia was identified as the protective predictor of mortality in the present study. This finding is compatible with several previous studies indicating lower mortality in patients with *E. coli* bacteremia originating from the urinary tract (19, 20). It may be attributed to the less severe clinical status, and most of our patients (82.8%) with bacteremia of urinary tract origin received active empirical antibiotic therapy.

Another question of practical interest is that of the effect of optimal levofloxacin PK/PD target attainment on clinical outcomes. The key prior cohort study showed that an AUC/MIC of ≥87 was a reasonable target, as determined by Monte Carlo simulation from MICs of 0.06 to 8 μg/ml, leading to a 4-fold significant effect on microbiological eradication without a link to clinical success (9). A possible explanation for the lack of a significant relationship between the PK/PD target and clinical outcomes may be the small number of patients, 47, included in the previous study (9). In another levofloxacin PK/PD study with various pathogens, a ratio of AUC to MIC of ≥110 (calculated from peak/MIC) resulted in bacterial killing and clinical cure (21). In the present study, the levofloxacin dosing and calculated levofloxacin drug level were comparable in the high- and low-MIC groups, but the ratio of estimated AUC to MIC was lower when the MIC increased, as one would expect. The calculated AUC/MIC of ≥87 and an AUC/MIC of ≥110 were found to have a correlation with clinical outcomes (Fig. 3B and Fig. S3). In addition, our study showed that the emergence of levofloxacin-resistant isolates was more common in the high-MIC group than the low-MIC group (Table 1). This trend indicates that the potential use of levofloxacin may lead to a suboptimal PK/PD target in the high-MIC group and result in the increased risk of resistant-strain acquisition.

The updated FQ breakpoints in the 2019 CLSI MIC criteria bring them into line with the European Committee on Antimicrobial Susceptibility Testing criteria of 2017, which

was recognized by the U.S. Food and Drug Administration on 17 June 2019 (22, 23). However, a more recent cross-sectional survey in the United States showed that only 24.3% of microbiology laboratories implemented the 2019 updated FQ breakpoints for *Enterobacterales*, and 97.3% of clinical laboratories used automatic systems to perform AST (24). There are no regulations that oblige manufacturers or clinical laboratories to adopt the revised breakpoints. However, our study confirmed the clinical impact of the revised levofloxacin breakpoints, and this suggests that clinical laboratories should endeavor to achieve consistency in AST performance, regardless of the AST method used, to ensure patient safety and favorable outcomes.

There were several limitations to the current study. First, the MICs were determined using the Vitek 2 system instead of by the CLSI-endorsed broth microdilution (BMD) reference method. It was difficult to distinguish the exact MIC within the range of a standard error of two doubling dilutions for MICs, and this would result in misclassification of whether an organism was levofloxacin susceptible or levofloxacin nonsusceptible, impacting outcomes. The previous study has demonstrated that the agreement between Vitek 2 and BMD was acceptable in *Enterobacterales*, although no specific information at levofloxacin MICs of $\leq$0.5, 1, and 2 μg/ml was provided (25). Second, because no blood samples for plasma levofloxacin concentration were available, an estimation of AUC was calculated using the population PK of levofloxacin in Korean patients (26), who shared similar baseline characteristics with our patients. Third, we are unable to comment on whether the diversity of the empirical antimicrobial agents administered and the different dosing regimens may have influenced the clinical outcomes. More patients infected by high-MIC isolates received inactive empirical antimicrobial therapy. However, the variable of empirical nonactive antibiotics was not associated with 30-day mortality (OR, 2.09; 95% CI, 0.61 to 7.20; $P = 0.240$) and was controlled between the low- and high-MIC groups in propensity score matching (Table S1).

In conclusion, the current study revealed that levofloxacin MICs within the historically susceptible range of 1 or 2 μg/ml in patients infected with *Enterobacterales* were associated with worse clinical outcome for mortality than MICs of $\leq$0.5 μg/ml. Our findings provide clinical evidence that the pre-2019 susceptibility breakpoints for levofloxacin were likely too high and thus support the revised criteria.

MATERIALS AND METHODS

Study design and population. A multicenter, retrospective observational cohort study was conducted at 3 institutions affiliated with Kaohsiung Medical University in southern Taiwan. A total of 2,400 beds were in one medical center and two regional hospitals. The study was reviewed and approved by the Institutional Review Board (IRB) of Kaohsiung Medical University Hospital [IRB no. KMUHIRB-E(II)-20200098].

Adult ($\geq$20 years of age) patients who had at least one positive blood culture report for *Enterobacterales* isolates between January 2017 and March 2019 were included in this study. If a patient experienced more than one episode of bacteremia, only the first episode was included. The day that the species was identified and AST results were obtained from the positive blood culture was defined as the index date. Eligible patients fulfilled the following criteria: (i) clinical symptoms or signs were compatible with sepsis syndrome, and (ii) levofloxacin had been administered for $>$72 h until the end of antimicrobial therapy. Patients were excluded if they (i) had bacteremia caused by *Enterobacterales* isolates with a levofloxacin MIC of $>$2 μg/ml; (ii) had polymicrobial bacteremia, i.e., a bacteremic episode due to at least two different organisms isolated from the same blood sample (27); (iii) expired before the index date; (iv) did not receive levofloxacin treatment on the index date as definitive therapy; or (v) received combination antibiotic therapy or were treated with levofloxacin for $\leq$72 h. Patient demographics, clinical and microbiological characteristics, antibiotic treatment, and clinical outcomes were retrieved from the medical records of eligible patients using a standardized case record form and reviewed.

Patients treated with levofloxacin were categorized into two groups: the low-MIC group and the high-MIC group. The low-MIC group consisted of patients infected with *Enterobacterales* with a levofloxacin MIC of $\leq$0.5 μg/ml. The high-MIC group consisted of patients infected with *Enterobacterales* with a levofloxacin MIC of 1 or 2 μg/ml. Therefore, all patients included in this evaluation were treated with levofloxacin on the index date as definitive therapy for $>$72 h and had isolates considered susceptible based on the MIC, according to the previous CLSI interpretive criteria (28).

Microbiological studies. The Bruker Biotyper (software version 3.0) matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) system (Bruker Daltonik GmbH, Leipzig, Germany) and the Vitek 2 system (bioMérieux, Marcy l'Etoile, France) were used for identification. The

levofloxacin MICs were determined using the Vitek 2 system. *In vitro* susceptibilities to levofloxacin were required to be quantified as MICs of ≤0.12, 0.25, 0.5, 1, 2, 4, or ≥8 μg/ml. The MICs were interpreted according to the breakpoints recommended by the CLSI in 2019 (4).

Variable definition and outcomes. The Charlson comorbidity index (CCI) was used to assess cases' comorbidities (29). Immunocompromised patients were defined as patients who received corticosteroid therapy (>10 mg/day) for more than 2 weeks or antineoplastic chemotherapy or immunosuppressive drugs 4 weeks before the onset of bacteremia (30). The sequential organ failure assessment (SOFA) score and the Pitt bacteremia score were utilized to grade the clinical severity of the infection on the day when levofloxacin was initiated (31, 32). Sources of bacteremia were assessed by three infectious disease physicians and one microbiologist and established according to the Centers for Disease Control and Prevention criteria for infection (33). Empiric therapy referred to antibiotics that were administered prior to the index date, whereas definitive therapy referred to the antibiotic therapy given after the receipt of AST results. An empirical antibiotic therapy other than levofloxacin was considered active depending on the susceptibility of the isolated strains to the selected antibiotics. Quarterly data on levofloxacin consumption were expressed as defined daily dose (DDD) and normalized per 1,000 patient-days. The creatinine clearance (CL_{CR}) was estimated using the modification of diet in renal disease equation (34). The area under the concentration-time curve (AUC) calculation was performed using the PK model of levofloxacin clearance: $6.19 \times (CL_{CR}/75)^{1.32}$ (26).

The primary outcome was 30-day mortality, and the secondary outcome was the incidence of emergence of levofloxacin-resistant isolates (sequential isolates with MICs of ≥4 μg/ml) within 90 days after levofloxacin initiation.

Statistical analysis. Statistical analyses were conducted using IBM SPSS version 20 (IBM Corp., Armonk, NY, USA) and the R software program, version 2.12 (R Foundation for Statistical Computing, Vienna, Austria). Categorical variables were compared using the chi-squared test or Fisher's exact test, as appropriate. Continuous variables were expressed as the mean ± standard deviation (SD) and compared using Student's *t* test. Spearman's correlation coefficient (ρ) was calculated for the association of antibiotic use and levofloxacin-resistant isolates. Event-time distributions were estimated using the Kaplan-Meier method. Variables yielding a *P* value of <0.05 in the univariable analysis were incorporated into the multivariable backward stepwise logistic regression analysis to identify factors that were significantly associated with mortality. Model adequacy was evaluated using the Hosmer-Lemeshow goodness-of-fit test and the area under the receiver operating characteristic (ROC) curve. The propensity score matching analysis was performed as a sensitivity test to verify the association between levofloxacin MIC and outcomes. A 1:3 nearest-neighbor propensity score matching without replacement was performed with a caliper width of 0.2. Patients in low- and high-MIC groups were matched on the basis of selected baseline variables, including intensive care unit (ICU) admission, immunocompromised status, a CCI of ≥3, a Pitt bacteremia score of ≥4, hospital-acquired infection, source of bacteremia, and presence of *Escherichia coli* isolates. All tests were two sided, and a *P* value of <0.05 was considered to indicate a statistically significant difference.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

ACKNOWLEDGMENTS

We have no conflicts of interest to declare.

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